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K Itoh; ... et. al

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EFFECT OF TUNICAMYCIN AND NEURAMINIDASE ON THE EXPRESSION OF Fc-IgM AND -IgG RECEPTORS ON HUMAN LYMPHOCYTES[1]

KYOGO ITOH AND KATSUO KUMAGAI

From the Department of Microbiology, Tohoku University School of Dentistry, Sendai, Japan

Using a rosette technique with ox red blood cells (ORBC) coated with rabbit IgG or IgM antibody, we have investigated the mode of expression of IgM-Fc and IgG-Fc receptors present on human lymphocytes in culture and the effect of neuraminidase and tunicamycin on their expression. The absolute number of cells bearing either IgM or IgG receptors when detected by highly sensitized ORBC did not differ before and after culture at 37°C. However, the number of cells with IgM receptors when detected by lightly sensitized ORBC was sharply increased soon after culture and that of IgG receptors was decreased inversely. IgM receptors were almost completely inactivated by *Vibrio cholerae* neuraminidase at concentrations of more than 0.02 units/ml and re-expressed after culture of cells at 37°C for 12 hr. In contrast, neuraminidase enhanced the expression of IgG receptors and the receptor expression enhanced by neuraminidase was reduced after culture. One to 10 μg/ml of tunicamycin, an antibiotic that inhibits protein glycosylation, inhibited the IgM receptor expression in culture. The antibiotic also blocked the reduction of IgG receptor expression in culture. Similar effects of neuraminidase and tunicamycin were observed with IgM and IgG receptors present on purified T cells. These studies demonstrate that the expressions of IgM- and IgG-Fc receptors on human T, and probably non-T, lymphocytes in culture even under nonstimulating conditions change in opposite directions and that protein glycosylation in the cell membrane may be a common factor influencing the expression of Fc receptors. This is necessary for the structure of IgM receptors but unnecessary for the structure of IgG receptors to exert a receptor activity.

In recent years receptors for immunoglobulin on the surface of human lymphocytes have been studied with a variety of techniques. Receptors for the Fc portion of IgG molecules have been detected on B and T cells, and on K and NK, populations that have not been characterized (1–5). In addition, receptors for the Fc portion of IgM (1, 6–10), IgA (11), or IgE (12) have been reported. The involvement of receptors for IgG in antibody-dependent cell-mediated cytotoxicity has been clearly recognized (1, 4, 5, 13, 14). However, the full significance of these receptors on lymphocytes is, in most cases, not yet understood. In the initial studies, IgM receptors were observed only on T cells (6), but later it has been shown that these receptors could be detected, under special conditions, on a certain proportion of B lymphocytes (15, 16). Within the T cell system intensive investigations have been carried out on the use of these receptors as a marker of a subset of T cells (Tμ)[2] different from that characterized by the IgG (Tγ). Several investigators have shown that Tμ cells provide help in a B cell immunoglobulin-producing assay (17, 18). Tγ cells, after interaction with IgG immune complexes, act as suppressor cells (17–19). On the other hand, it has been reported that IgG and IgM receptors do not appear to be markers for distinct T cell subsets under some conditions, and rather a subset of T cells may conceivably express both classes of receptors at different functional stages (1, 8).

IgM receptors usually cannot be detected on freshly isolated cells, but can be on cells that have been cultured overnight in IgM- free media (6, 15, 16). This special requirement for IgM receptors has been reported to be related possibly to the high avidity of the receptor for the substrate (6, 7, 20). In contrast, the highest IgG receptor values are obtained immediately after isolation of the cells, before culture at 37°C. After culture, there is a drop of IgG receptor values (8, 16). It has been shown that the spontaneous decrease of cells with IgG receptors may be due, in part, to shedding of receptor molecules from the cell (8, 16). However, the factors related to the expression of both receptors in culture are not yet clearly established. In the present study, we have therefore reinvestigated the expression of the IgM and IgG receptors on human lymphocytes as a function of time in culture and studied the effect of neuraminidase and tunicamycin (TM) on the IgM and IgG receptor expressions.

MATERIALS AND METHODS

Preparation of lymphocytes. Human peripheral blood mononuclear cells from healthy volunteers were prepared by the techniques of Ficoll-Isopaque gradients as described previously (3, 21). For assays of Fc receptors and culture experiments, lymphocyte preparations depleted of monocytes by adherent

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[2] Abbreviations used in this paper; Tμ, T cells bearing Fc receptors for IgM: Tγ, T cells bearing Fc receptors for IgG: ORBC, ox red blood cells; EAμ, ox red blood cells coated with IgM antibody; EAγ, ox red blood cells coated with IgG antibody; En, sheep red blood cells treated with neuraminidase; En-RFC, En-rosette-forming cells; EAμ-RFC, EAμ-rosette-forming cells; EAγ-RFC, EAγ-rosette-forming cells; TM, tunicamycin.

techniques were always used (22). Approximately 1 or 2 × 10^7 of mononuclear cells suspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (RPMI/10% FCS) were incorporated into a plastic Petri dish (6 x 6 cm in diameter, Falcon, Oxnard, Calif.) and incubated for 1 hr at 37°C to attach the monocytes. After incubation, nonadherent cells were collected, washed by centrifugation, and suspended in RPMI/10% FCS. This method generally yielded lymphocyte preparations containing less than 5.0% monocytes as judged by the acid α-naphthyl acetate esterase activity or phagocytosis of yeast particles (22).

Cell cultures. Cells were cultured at a concentration of 1 × 10^6 cells/ml in 5% CO_2/95% air at 37°C in plastic tubes. RPMI/10% FCS was used in all the experiments.

Detection of Fc receptors. Ox red blood cells (ORBC) coated with IgG (EAγ) or IgM (EAμ) were prepared essentially as described by Meretta *et al.* (6). Anti-ORBC serum was prepared in rabbits by i.v. immunizations with ORBC. An IgM-rich immune serum was obtained from the rabbits 1 or 2 weeks after a single administration of ORBC. IgG-rich hyperimmune sera were prepared from the rabbits that received a successive administration of ORBC once a week for 5 weeks. The IgG and IgM fractions were obtained by precipitation with 33% ammonium sulfate followed by Sephadex G-200 (Pharmacia, Uppsala, Sweden) gel filtration. These were further purified by a rabbit IgM or IgG immunoadsorbent column. The purity of these fractions were tested by immunoelectrophoresis. The EAγ was prepared with ORBC coated with serial dilutions of 1/20 diluted anti-ORBC IgG fraction (protein concentration, 10 mg/ml and hemolysis titer, 1:20,000) at 37°C for 30 min. The EAμ was prepared by incubation of ORBC with serial dilutions of anti-ORBC IgM fraction (protein concentration, 0.8 mg/ml; hemolysis titer, 1:20,000) at 37°C for 90 min. These EAγ and EAμ thus obtained were washed three times with phosphate-buffered saline, pH 7.2 (PBS), resuspended in RPMI/10% FCS, and stored at 4°C until use. The lymphocytes were washed twice and resuspended in RPMI 1640 medium containing 1% fetal calf serum (RPMI/1% FCS). One-tenth milliliter of the cells (1 × 10^6/ml) and equal volume of 1% sensitized ORBC were mixed, spun down at 1,000 rpm for 10 min at 4°C, and then kept at 4°C for 1 to 3 hr. After incubation, mixtures were resuspended very gently by pipetting. A drop of resuspended cells was placed on a hemocytometer and at least 250 cells were counted. Adherence of three or more erythrocytes was considered positive. Uncoated ORBC served as controls. In all instances, the percentage of rosetting cells recorded represented the mean of two independent determinations. The specificity of the receptors for IgM and IgG was assessed by blocking experiments of EAμ and EAγ rosettes with purified human IgM and IgG or Fc-piece purified from human IgG and myeloma protein of the IgM type.

All data are expressed as the mean values and the standard deviations (S.D.) of duplicate cultures in one representative experiment obtained from the same donor unless otherwise stated.

Detection of En-rosette forming cells. Spontaneous rosette formation between human lymphocytes and sheep red blood cells (SRBC) was assayed with neuraminidase-treated SRBC (En) as previously described (7, 8).

Purification of T cells. T cells were separated from non-T cells according to their capacity to form rosettes with En (En-RFC), as previously described (7, 8). Briefly, monocyte-depleted human peripheral blood mononuclear cells were mixed with En, spun at 1,000 rpm for 10 min, and incubated at 4°C for 1 hr. The En-RFC (T) cells were separated from the nonrosette-forming (non-T) cells by Ficoll-Isopaque centrifugation at 2,000 rpm for 30 min. If the enrichment of the En-RFC cells was not satisfactory, the same procedure was repeated. The En-RFC were dissociated by hypotonic shock. These fractionation procedures gave a cell suspension containing 96 to 98% En-RFC.

Neuraminidase-treatment of cells. Neuraminidase (from *Vibrio cholerae*) was purchased from Behringwerke, A. G., Marburg, Federal Republic of Germany. The original solution (1 U/ml) was serially diluted in PBS. Of these solutions, 0.1 ml was added to 1.0 ml of freshly prepared or cultured lymphocyte suspensions (1 × 10^6 cells/ml). The cell suspensions were incubated at 37°C for 30 min. The enzyme-treated cells were tested either directly or after incubation at 37°C for the expression of Fc receptors. Final concentrations of neuraminidase tested were 0.01 to 0.1 U/ml. At these concentrations, the lymphocytes of more than 95% were viable as judged by trypan blue exclusion tests.

TM treatment. TM (23), kindly supplied by G. Tamura, Department of Agricultural Chemistry, University of Tokyo, was added to the lymphocyte cultures at concentrations ranging from 0.1 to 10 μg/ml. *N*-acetylglucosamine (Wako Chemical Co., Japan) was used as an inhibitor of TM action.

RESULTS

Expression of IgM and IgG receptors in culture. First, we examined the mode of expression of IgM and IgG receptors on the freshly isolated lymphocytes in culture at 37°C by using the erythrocytes sensitized with serial dilutions of anti-ORBC IgM and IgG antibodies. The representative results obtained with a healthy donor given in Figure 1 show that the IgM receptor-bearing cells, when detected by EAμ sensitized with low concentrations of antiserum, increased sharply with time, particularly for the initial several hours after culture, and then reached the maximum at around 20 hr. When assayed by using a high concentration of IgM antibody (800 μg/ml), however, the values near the maximum of IgM receptors on the lymphocytes were detected even before culture.

The results depicted in Figure 2 show that the values of IgG receptor-bearing cells detected before culture varied depending

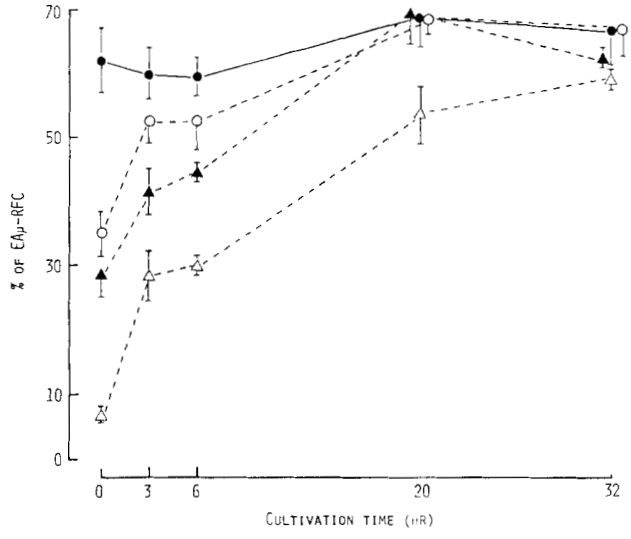

Figure 1. Expression of IgM receptors on human lymphocytes in culture as a function of time. Values represent the rosette-forming cells detected by ORBC sensitized with rabbit anti-ORBC IgM at 800 (●——●), 270 (○-----○), 90 (▲-----▲), and 30 (△-----△) μg/ml.

on the concentrations of antiserum used for sensitization of EA. The cell numbers when detected by a high concentration of IgG antibody (500 μg/ml) decreased only slightly after overnight cultivation, whereas those when detected by lower concentrations of antibody sharply decreased for an initial several hours. The experiments were carried out in the other individuals with similar results.

These results indicate that the absolute number of cells bearing IgM or IgG receptors may not change substantially in culture, since both cell types could be detected at or near maximum values either before or after culture when examined by the highly sensitized indicator cells. The results, however, indicate that the avidity of IgM receptors may increase in culture and that of IgG receptors may decrease inversely, and that both changes may occur in parallel very soon after culture, go on most actively for an initial several hours, and continue until 18 or 20 hr.

Effect of neuraminidase-treatment on the IgM and IgG receptors. Next, we examined the susceptibility of both receptors to neuraminidase treatment. Freshly prepared lymphocytes were treated with different concentrations of neuraminidase at 37°C for 30 min. The percentages of cells bearing receptors for IgM or IgG were determined by using ORBC sensitized with a high concentration of IgM (800 μg/ml) or IgG (500 μg/ml) antibody before and after the treatment. As shown in Figure 3, IgM receptors were extremely sensitive to neuraminidase; the receptors were inactivated by neuraminidase even at a concentration of 0.02 unit/ml and completely removed from the cells treated with 0.05 units/ml or more of the enzyme. In contrast, the number of cells with IgG receptors was significantly increased after treatment with neuraminidase (at 0.01 unit/ml) and reached the maximum in the cells treated with 0.05 unit/ml or more of the enzyme. These results clearly indicate that neuraminic acid in the cell membranes may be required for the structure of IgM receptors to exert a receptor activity and inversely inhibit that of IgG receptors.

Effect of TM on the expression of IgM and IgG receptors. As a consequence of the data that the increasing expression in culture and high susceptibility to neuraminidase of IgM receptors were in a striking contrast to the decreasing expression in culture and augmented expression by neuraminidase of IgG receptors, it is conceivable that the expression of both receptors on the cell are regulated by glycosylation of cell membrane proteins. Thus, we examined whether TM, a specific inhibitor of glycosylation of cellular proteins by blocking the formation

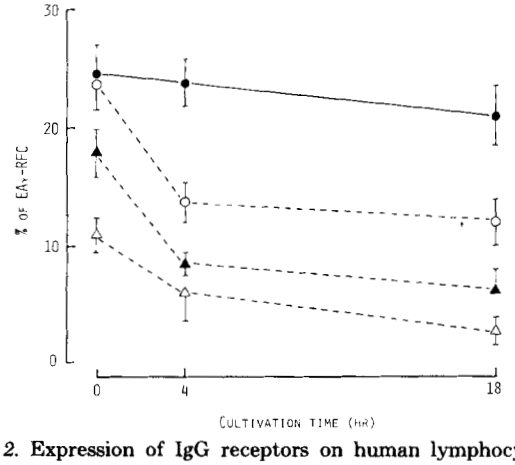

Figure 2. Expression of IgG receptors on human lymphocytes in culture as a function of time. Values represent the rosette-forming cells detected by ORBC sensitized with rabbit anti-ORBC IgG at 500 (●——●), 180 (○-----○), 60 (▲-----▲), and 20 (△-----△) μg/ml.

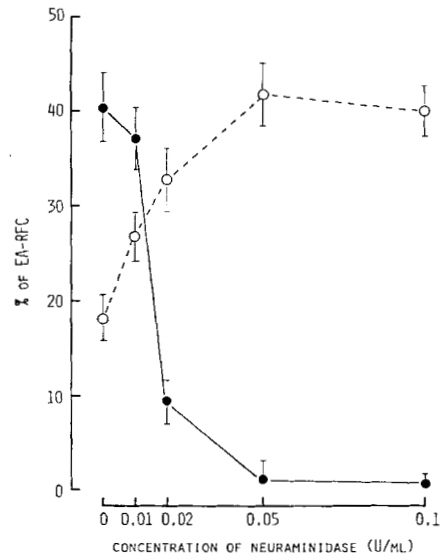

Figure 3. Effect of neuraminidase on IgM and IgG receptors present on human lymphocytes. Cells were suspended in PBS containing different concentrations of neuraminidase and incubated at 37°C for 30 min. IgM receptors (●——●) and IgG receptors (○-----○) were detected by ORBC sensitized with IgM antibodies at 800 μg/ml and IgG antibody at 500 μg/ml, respectively.

of *N*-acetylglucosamine-lipid-intermediates (23), can affect the expression of IgM and IgG receptors in culture. Freshly isolated lymphocytes were cultivated at 37°C in the presence of 0.1 to 10 μg/ml TM and then examined for IgM receptors by using EAμ sensitized with a suboptimal concentration of IgM antibody (90 μg/ml). The results (Fig. 4) clearly show that the progression of IgM receptor expression in culture was completely inhibited by the addition of 10 and 1 μg/ml but not 0.1 μg/ml of TM. Even when examined with EAμ sensitized with high concentrations of antibody (270 to 800 μg/ml), a significant but incomplete inhibitory effect of the antibiotic on the expression of IgM receptors was observed at 24 hr of culture (data not shown). Figure 4 also shows that the expression of IgM receptors progressed normally in the culture when we added 1.0 μg/ml of TM and 10^{-3} M *N*-acetylglucosamine, a specific inhibitor of TM action. Addition of 10^{-3} M *N*-acetylglucosamine alone did not have any effect on the expression of IgM receptors (data not shown).

Next, cells, after overnight culture in which IgM receptors were fully expressed, were treated with 0.1 unit/ml neuraminidase and recultivated at 37°C in the presence or absence of TM. The effect of TM on the expression of IgM receptors in culture was then examined by using EAμ sensitized with an IgM antibody at 90 μg/ml. As clearly shown in Figure 5, the IgM receptors removed by neuraminidase were reexpressed soon after recultivation of cells in the absence of TM, and the number of cells with the receptors returned within 12 hr to the values before neuraminidase treatment. In contrast, in the presence of 1.0 μg/ml TM, the reexpression of IgM receptors after culture was remarkably inhibited, whereas that of IgM receptors in the presence of 1.0 μg/ml TM and 10^{-3} M *N*-acetylglucosamine progressed normally. Freshly isolated cells treated with neuraminidase, in which IgG receptors were fully expressed, were also cultured in the presence of TM alone or TM and *N*-acetylglucosamine. The effect of TM on the spontaneous decrease of IgG receptors in culture was then examined. As shown in Figure 6, the number of cells with IgG receptors decreased with time either in the control cultures or the culture

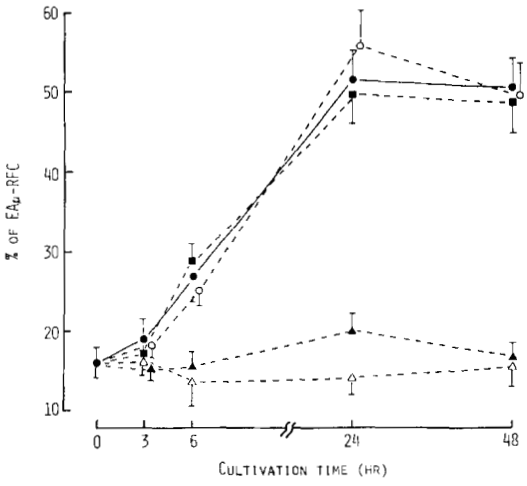

Figure 4. Effect of TM or TM and *N*-acetylglucosamine on expression of IgM receptors present of human lymphocytes in culture. The IgM receptor-bearing cells were detected by ORBC sensitized with IgM antibody at 90 μg/ml. The values represent the means ± S.D. of triplicate cultures of lymphocytes with 0.1 μg (○-----○), 1 μg (▲-----▲) or 10 μg (△-----△) per ml of TM, with 1 μg/ml of TM and 10^{-3} M (220 μg/ml) *N*-acetylglucosamine (■-----■), or without either substance (●——●).

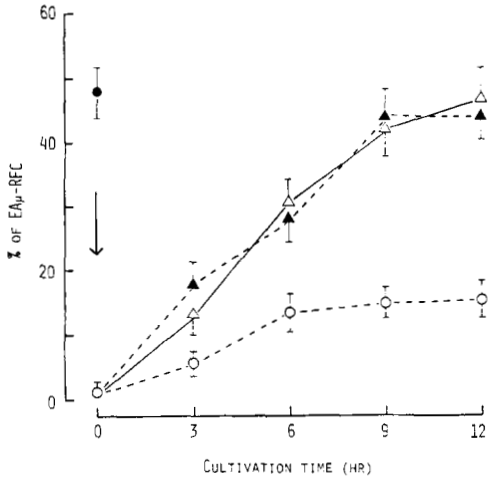

Figure 5. Reexpression of IgM receptors on lymphocytes treated with neuraminidase and effect of TM on reexpression. Cells that had been cultured overnight were treated with 0.1 units/ml of neuraminidase and then recultivated in the presence of 1 μg/ml TM (○-----○), 1 μg/ml TM and 10^{-3} M *N*-acetylglucosamine (▲-----▲), or in the absence of either substance (△——△). ●, represents the values of cells with IgM receptors before neuraminidase-treatment of cells. IgM receptors were detected by EAμ sensitized with IgM antibody at 90 μg/ml. The values represent the means and S.D. of four cultures from the same subject.

with TM and *N*-acetylglucosamine, in contrast, those cultivated in the presence of TM alone remained constant.

These results indicate that the effect of TM on the expression of IgM and IgG receptors may be specifically related to its inhibitory effect on glycosylation, and that the glycosylation is necessary for the expression of IgM receptors, but may be inhibitory for the expression of IgG receptors.

Susceptibility to neuraminidase and TM of IgM and IgG receptors on purified T cells. T-enriched cells were isolated as described above and then examined for the susceptibility to neuraminidase and TM treatments of IgM and IgG receptors on the cell. As shown in Table I, the expression of IgM receptors on purified T cells was easily inhibited by neuraminidase and that of IgG receptors was enhanced inversely, as were those on unpurified lymphocytes. Table II shows that the expression of IgM receptors on purified T cells was inhibited by TM, as was that on unpurified lymphocytes, and that the inhibition of receptor expression by TM was blocked by *N*-acetylglucosamine.

DISCUSSION

Initial studies of IgM receptors on human lymphocytes have shown that the overnight cultivation of cells at 37°C is required for the expression of IgM receptors (6, 15, 16, 20). Later, it was shown that freshly isolated T cells begin to express IgM receptors *in vitro* within an incubation time as short as 1 or 2 hr and that maximum expression of these receptors is observed after 6 hr (7). As to the IgG receptors, it has been reported that the

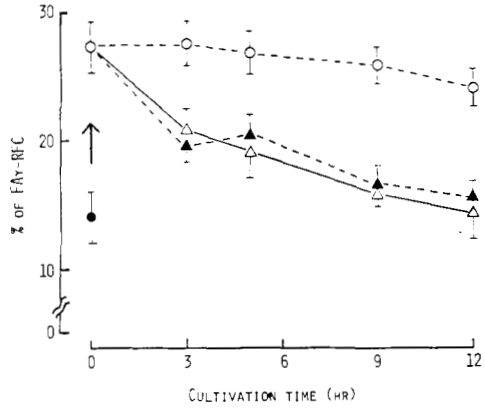

Figure 6. Effect of TM on the spontaneous decrease of cells with IgG receptors in culture. Cells treated with 0.1 units/ml of neuraminidase were cultivated in the presence of 1 μg/ml TM (○-----○), 1 μg/ml TM and 5×10^{-4} M *N*-acetylglucosamine (▲-----▲), or in the absence of either substance (△——△). ●, represents the values of cells with IgG receptors before neuraminidase-treatment of cells. IgG receptors were detected by EAγ sensitized with IgG antibody at 500 μg/ml. The values represent the means and S.D. of four cultures from the same subject.

TABLE I

Effect of neuraminidase treatment on IgM and IgG receptors present on purified T cells[a]

Treatment with Neuraminidase (0.1 unit/ml)	% EAμ-RFC (±S.D.)			% EAγ-RFC (±S.D.)		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
−	80.2 (5.1)	56.0 (4.0)	67.3 (4.5)	11.9 (1.2)	14.6 (1.3)	10.0 (1.5)
+	1.2 (0.1)	0.5 (0.1)	1.0 (0.2)	20.0 (0.9)	21.4 (1.4)	18.0 (2.0)

[a] Purified T cells were cultured overnight in RPMI/10% FCS, treated with neuraminidase, and then tested for the percentages of EAμ- and EAγ-RFC by ORBC sensitized with IgM (800 μg/ml) and IgG (500 μg/ml) antibodies, respectively.

TABLE II

Effect of TM on the expression of IgM receptors present on purified T cells[a]

Reagents	% EAμ-RFC (±S.D.)		
	0 hr	12 hr	24 hr
None	24.9 (2.1)	55.1 (4.3)	67.7 (2.2)
TM 1 μg/ml		14.0 (1.1)	17.1 (0.9)
TM 1 μg/ml plus *N*-acetylglucosamine 10^{-3} M		52.3 (3.2)	65.1 (4.1)

[a] T cells purified from freshly isolated lymphocytes were cultured at 37°C in RPMI/10% FCS with TM or TM and *N*-acetylglucosamine. Receptors were detected by EAμ sensitized with 90 μg/ml of IgM antibody.

cells bearing the receptors decrease occasionally in culture (8, 16). The present results show that IgM receptors at levels near the maximum values can be detected even before culture when tested by using high concentrations of IgM antibody. The rapid increase of cells bearing IgM receptors was demonstrated only when tested by low titers of antibody. The cells bearing receptors for IgG at levels near the maximum values could be detected even after culture, although when tested by low titers of antibody, a rapid decrease in the values of cells bearing receptors was observed. Thus, it appears that the numbers of cells with IgM or IgG receptors may not differ before and after cultivation of cells.

The reasons for special requirement of exposure times for the full expression of IgM receptors may be due to the fact that the receptors on freshly isolated lymphocytes may be normally occupied by IgM molecules from the serum, and therefore, the expression of receptors needs exposure times until the receptors are shed in the media together with the bound IgM and the cultured cells resynthesize new receptors (6, 7, 20). However, the present results show that IgM on the highly sensitized EAμ could bind with high avidity to either freshly isolated or cultured cells competitively with endogenously bound IgM molecules on the receptors and that the values of IgM receptor-bearing cells detectable before culture were dependent upon the concentrations of antibody bound to EAμ. On the other hand, the spontaneous decrease of cells with the Fc-IgG receptor may be due to shedding of Fc-IgG receptors from the cell (8, 16). Indeed, the receptors, to which the immune complexes have been bound, are shed from the cell (8). However, the present results show that the values of IgG receptor-bearing cells after or in culture also were dependent upon the concentrations of antibody bound to EAγ and that IgG on the highly sensitized EAγ could bind even to cultured cells. These results may indicate that the avidity or number of IgM receptors present per cell increased in culture and that of IgG receptors decreased inversely, whereas the absolute number of cells bearing each receptor did not change in culture. The present results also show that the number of cells with IgM receptors when detected by lightly sensitized EAμ began to increase at the same time that the number of cells with IgG receptors began to decrease. Both changes occurred with a short incubation time of 1 or 2 hr. This may indicate that the IgM- and IgG-receptor expressions in culture are regulated by the same mechanisms by which each of the receptors changes in opposite directions, thus making unlikely the hypothesis that shedding and resynthesis of the receptors directly cause the variations of IgM- and IgG-receptor expressions in culture under nonstimulating conditions. To elucidate the factors regulating the changes, we have examined the effect of various enzymes and metabolic inhibitors on the expression of each receptor. We found that neuraminidase, an enzyme removing neuraminic acid from the cell, and TM, an antibiotic inhibiting the glycosylation of cellular proteins, affected the expression of IgG receptors and that of IgM receptors in opposite directions.

The expression of receptors for IgM was completely inhibited by low concentrations of neuraminidase, whereas that for IgG was augmented. Augmentation of IgG receptor expression by neuraminidase is consistent with the report of Haegard (24), but an extremely high susceptibility of IgM receptors to the enzyme is contrary to the report in which the not-inhibiting but rather enhancing effect of neuraminidase on the receptor was observed. The reason for this discrepancy remains unknown. However, it may depend on the IgM antibody used; in his report no IgM receptor-bearing cells were detected on freshly isolated lymphocytes, whereas in our study the number of cells near the maximum value could be detected. It is well known that removal of neuraminic acid from the cell results in revealing the hidden structural determinants of the cell surface (25, 26) or in destroying the apparent determinants (27–29). Thus, it appears that neuraminic acid in the cell membranes plays a role not only in masking the structures of some determinants, but also in keeping the structure of others to exert the activity. The present results indicate that neuraminic acid moiety may be required for the structure of IgM receptors to exert a receptor activity, although it remains undetermined whether neuraminic acid interacts directly with a receptor site for IgM or keeps indirectly the receptor site on the cell surface in such a configuration that it can interact with the receptor. It seems that neuraminic acid inversely masks the structure of IgG receptors, although it also remains undetermined how neuraminic acid interact with IgG receptors to hide the receptor structure.

TM, an antibiotic that prevents glycosylation of glycoproteins by blocking the formation of *N*-acetylglucosamine-lipid intermediates, is known to reduce the production of various cytoplasmic and membrane glycoproteins, such as fibronectin (30), collagen (31), or IgM and IgA (32). The antibiotic apparently inhibits the expression of IgM receptors during the cultivation and blocks the reduction of IgG receptor expression. It was also shown that the effect of TM on the expression of IgM and IgG receptors may be specifically related to its inhibitory effect on glycosylation. Thus, it may be concluded that glycosylation is necessary for the expression of IgM receptors, but is not necessary, but rather inhibitory for the expression of IgG receptors. All processes of glycosylation of membrane proteins cannot be inhibited by TM (30–34). Therefore, the possibility cannot be excluded that the IgG receptors also requires glycosylation of cell membrane proteins for their structure to exert a receptor activity. Both IgM and IgG receptors were impaired by the addition of 2-deoxy-D-glucose (unpublished observation). However, since 2-deoxy-D-glucose is known to have multiple metabolic effects within the cells (32, 35, 36), its inhibitory effect may not be specifically related to its effect on glycosylation.

The same effects of neuraminidase and TM were observed with IgM and IgG receptors present on purified T cells. These results indicate that modification of IgM and IgG receptors by glycosylation of cell membrane proteins may be a phenomenon observed in common with T and non-T lymphocytes.

It is known that IgM contains three to five carbohydrate units per heavy chain, whereas IgG contains only a single carbohydrate unit on its heavy chain (37). It is possible that the specific requirement of carbohydrate moiety for the IgM receptor may be related to the high quantity of carbohydrates on the heavy chain of IgM. No information is available for the need of carbohydrates in IgM molecules for binding to receptor sites. If this is the case, both the IgM Fc-site and the receptor site on the cell may require carbohydrates in those molecules for their specific binding, as is the case for specific binding of some virus to receptor sites on the cell (38).

Glycosylation may not be the only factor that influences the structure of receptor site for either IgM or IgG. Proteinase, such as trypsin or pronase, at low concentrations can easily inactivate the IgM receptors (7, 8). IgM receptors removed by pronase were reexpressed after cultivation at 37°C as were those inactivated by neuraminidase. The reexpression of IgM receptors removed by trypsin was not inhibited by the addition of TM, suggesting that there may also be a protein moiety

required for the structure of IgM receptors to exert a receptor activity (unpublished observation). IgM and IgG receptors were also different in susceptibility to phospholipase B and C (unpublished observation). Therefore, the structure of IgG and IgM receptors to exert a receptor activity may be much more complex since a number of moieties, such as protein, phospholipid, or carbohydrate modulate the receptor sites for IgM and IgG. To understand completely the difference of both receptor molecules, future studies, such as characterization of receptors isolated from the cell, will be needed. Neuraminidase and TM presented in this report may provide a useful tool in these studies.

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